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14. ABSTRACT

Based upon the results obtained from these studies, we can draw the following conclusions: 1) Airway hyperresponsiveness developed in Ova-sensitized mice was less pronounced in TRPV1-null mice, indicating an important role of TRPV1. 2) An increase in airway temperature within the normal physiological range triggered bronchoconstriction in sensitized rats, but not in control rats. Chronic airway inflammation in sensitized animals is likely a major contributing factor in causing this response. 3) transient increase in airway resistance was generated immediately after hyperventilation with warm humid air in patients with mild asthma, but the same warm humid air challenge failed to cause any bronchoconstriction in healthy subjects. Furthermore, this bronchoconstriction is likely generated by the increase in airway temperature because hyperventilation with humidified air at room temperature did not generate any change in airway resistance in the same patients. These studies, once completed, should provide important and novel information for: 1) documenting the pulmonary stresses induced by hyperthermia in healthy individuals and in patients with sensitized airways; 2) understanding the mechanism underlying the hyperthermia-induced pulmonary dysfunction; and 3) detecting the susceptibility to heat stress in soldiers with underestimated or overlooked airway hypersensitivity such as in airway allergy or mild asthma.

15. SUBJECT TERMS
Hyperthermia, asthma, airway constriction, cough, dyspnea

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INTRODUCTION

The working hypothesis of this TATRC project is that the expression of the transient receptor potential vanilloid type 1 (TRPV1) channel is up-regulated in the airway mucosa of patients with mild asthma, allergic rhinitis and upper respiratory infection, which makes these patients more susceptible to the bronchoconstriction and other respiratory dysfunctions induced by thermal stress.

There are four specific aims for the third year of this translational project: 1) To determine whether the acute bronchoconstrictive effect caused by airway hyperthermia was amplified by chronic airway allergic reaction. 2) To determine if thermal stress generated various airway dysfunctions (airway constriction, cough, etc.) in patients with allergic rhinitis. 3) To determine if thermal stress generated various airway dysfunctions in patients with laryngopharyngeal reflux. 3) To compare the responses observed in the study series 2) and 3) with that in healthy volunteers.

BODY

In the last twelve months, we have made major research progresses in this project. Two study series have been completed, five manuscripts have been published or accepted for publication, and two other study series are still in progress. Reprints of these full papers are submitted in the Appendix, and therefore only short summaries are presented here:

Task 1-2: To determine if bronchoconstriction induced by hyperventilation of humidified hot air in an animal model of asthma (ovalbumin-sensitized Brown Norway rats); and

Task 1-3: To determine the role of TRPV1 in the augmented airway response to hyperthermia in Ova-sensitized rats.

The manuscript reporting the results obtained frim this study has been accepted for publication by the Journal of Applied Physiology , a flagship journals of the American Physiological Society. Another manuscript will be submitted for publication in the same journal in December or January 2014.

Summary 1: The proposed study was carried out to determine the effect of allergic inflammation on the airway response to increasing airway temperature. Our results showed: 1) In Brown-Norway rats actively sensitized by ovalbumin (Ova), isocapnic hyperventilation with humidified warm air (HWA) for 2 min raised tracheal temperature (T_{tr}) from 33.4 ± 0.6 to 40.6 ± 0.1°C, which induced an immediate and sustained (>10 min) increase in total pulmonary resistance (R_L) from 0.128 ± 0.004 to 0.212 ± 0.013 cmH_2O/ml/s (n=6, P<0.01). In sharp contrast, the HWA challenge caused the same increase in T_{tr}, but did not generate any increase in R_L in control rats. 2) The increase in R_L in sensitized rats was reproducible when the same HWA challenge was repeated 60-90 min later. 3) This bronchoconstrictive effect was temperature dependent; a slightly smaller increase in peak T_{tr} (39.6 ± 0.2°C) generated a significant but

smaller increase in R_L in sensitized rats. 4) The HWA-induced bronchoconstriction was not generated by the humidity delivered by the HWA challenge alone because the same water content delivered by saline aerosol at room temperature had no effect. 5) The HWA-evoked increase in R_L in sensitized rats was not blocked by atropine, but was completely prevented by a pretreatment with either a combination of neurokinin (NK)-1 and NK-2 antagonists, or with formoterol, a β_2 agonist, before the HWA challenge. This study showed that increasing airway temperature evoked a pronounced and reversible increase in airway resistance in sensitized rats and tachykinins released from the vagal bronchopulmonary C-fiber endings were primarily responsible.

Summary 2: Our recent study supported by this TATRC contract has reported that hyperventilation of humid warm air (WA) triggered cough and reflex bronchoconstriction in patients with mild asthma (Am. J. Resp. Crit. Care Med. 185:1190-96, 2012). We suggested that sensitization of the bronchopulmonary C-fibers by increasing airway temperature is involved, but direct evidence is lacking. This study was carried out to determine whether hyperventilation of WA enhanced the pulmonary C-fiber sensitivity in an animal model of asthma (Ova-sensitized Brown-Norway rats). Isocapnic hyperventilation of WA for 3 minutes rapidly raised airway temperature to a peak of ~42℃, which significantly elevated the base-line fiber activity (FA) of pulmonary C-fibers in Ova rats immediately after the termination of WA challenge, but not in the control rats (Figures 1 & 2). Furthermore, the pulmonary C-fiber response to right atrial injection of capsaicin was significantly higher in Ova rats than control rats before the WA challenge; this difference in the FA response to capsaicin was further amplified after WA in Ova rats (Δ=4.98±1.78 imp/s before, and 10.34±2.21 imp/s after WA; $P<0.05$, n=9) (Figures 1 & 3). A similar pattern of the WA-induced potentiation of the FA response to phenylbiguanide was also founded in Ova rats (Figure 4). These results suggest that Ova-sensitization increases the excitability of pulmonary C-fibers to both TRPV1 and non-TRPV1 activators. The hypersensitivity is further enhanced by an increase in the airway temperature.

Tasks 2-1, 2-3 & 3-3: Airway stress induced by hyperventilation of humidified hot air in patients with allergic rhinitis.

Results obtained from this study series were presented in an abstract in the 2013 American Thoracic Society International Conference, and have also been submitted as a full manuscript to the journal of Clinical and Experimental Allergy, which is currently under review.

Summary 3: We hypothesized that chronic inflammation results in up-regulation and sensitization of TRPV1 receptors in the upper airways of patients with allergic rhinitis (AR). We carried out the following pilot study to test this hypothesis. Airway resistance (Raw), spirometry indices, and numbers of coughs were recorded prior, during and after isocapnic hyperventilation of both humidified HA and RA for 4 minutes (protocol identical to that described above in the study of asthmatics) in 7 patients (2 male, 5 female) with moderate allergic rhinitis and 6 healthy subjects (3 male, 3 female). Subject characterization and AR symptom severity assessments are

presented in Table 1. Our results showed that the HA challenge consistently triggered cough (recorded by a Vitalograph cough monitor) in the AR patients, but not in healthy subjects (e.g., Figure 5). In AR patients, number of coughs increased from 0.10 ± 0.07 at baseline to 2.37 ± 0.73 during and to 1.80 ± 0.79 (coughs/min) after the isocapnic hyperventilation of HA ($p<0.01$, $n=7$). In sharp contrast, the same HA hyperventilation challenge did not cause any significant tussive effect in healthy subjects (Figure 6). Hyperventilation of RA did not generate any significant change in number of coughs in either AR or healthy individuals (Figures 5 & 6). Furthermore, the AR subjects expressed significantly more respiratory discomfort via hand grip dynamometry as compared to healthy subjects (Figure 7). However, airway resistance did not significantly increase after the HA in AR patients (Figure 8), in contrast to that observed previously in asthmatics. In conclusion, hyperventilation of humid HA triggered vigorous cough response and respiratory discomfort in AR patients, indicating the involvement of the airway sensory nerves. Chronic inflammation in the upper airways may have contributed to an up-regulation of the sensitivity and/or expression of TRPV1 in these sensory nerves. Further studies are required to test this hypothesis.

Tasks 2-1, 2-4, & 3-4: Cough hypersensitivity induced by breathing hot humid air in patients with laryngopharyngeal reflux: a possible role of TRPV1.

Summary 4: Laryngopharyngeal reflux (LPR) has been identified as one of the most common diseases associated with chronic non-productive cough. Expression of TRPV1 has been recently reported in the human larynx. We therefore tested the hypothesis that chronic inflammation induces over-expression of TRPV1 in laryngeal C-fiber afferents in LPR patients, and breathing hot humid air can activate TRPV1 and elicit cough in these patients. Cough was recorded with a Vitalograph cough monitor and the adductor motion of vocal folds recorded by a flexible nasopharyngoscope inserted through nare. In three patients with LPR and one healthy control, vigorous cough response accompanied by high frequency of vocal fold adductor motion were triggered during hot humid air inhalation, which gradually declined after the challenge. These responses were absent in the control subject.

This study is currently in progress; we will continue the study on four additional LPR aptients and six additional matching healthy subjects during the no-cost-extension of this TATRC project.

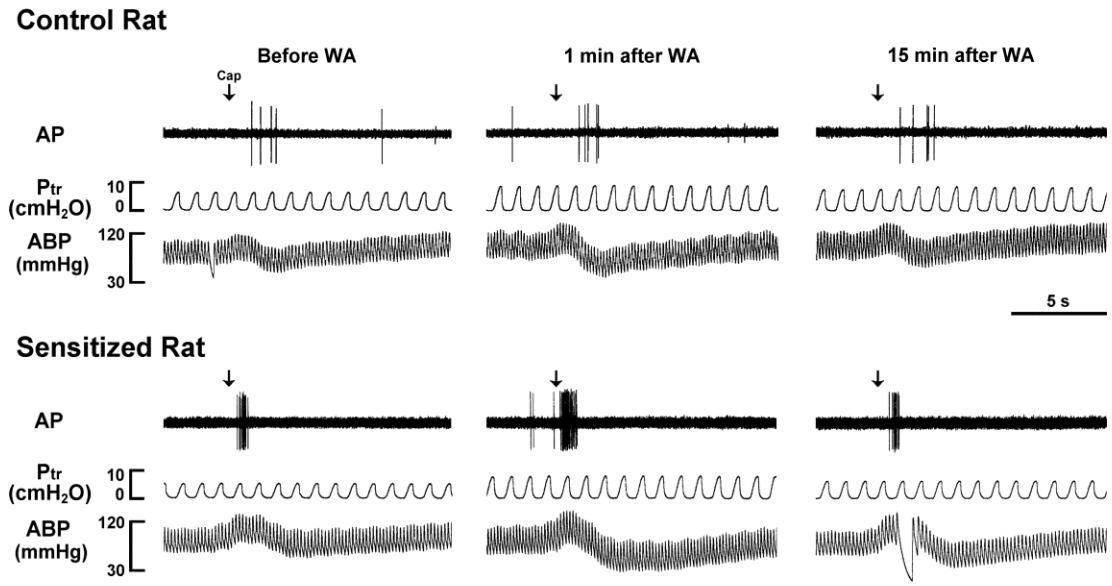

Fig. 1. Experimental records illustrating the effects of WA challenge on the pulmonary C-fiber responses to capsaicin (Cap, 0.75 mg/kg) in anesthetized, closed-chest, and artificially ventilated rats. From left to right: responses to Cap before, at 1 min and 15 min after WA challenge, respectively. AP, action potential; Ptr, tracheal pressure; ABP, arterial blood pressure. Receptor location in the control rat (260 g): right upper lobe. Receptor location in the sensitized rat (290 g): right accessory lobe.

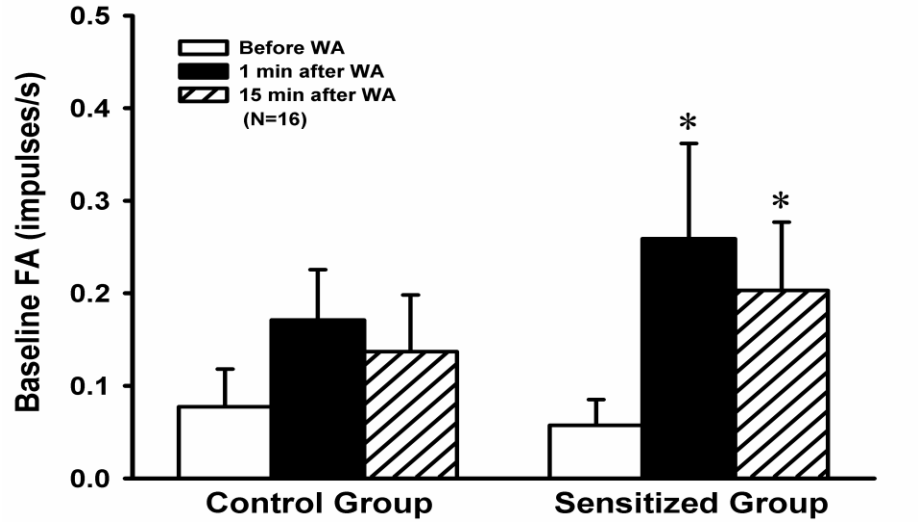

Fig. 2. A comparison of the average baseline fiber activities (FA) before, at 1 min and 15 min after WA challenge between control and sensitized rats. Baseline FA was averaged over 20 s in each fiber. Data are means ± SE of 16 rats in each group. *P<0.05, significantly different from before WA.

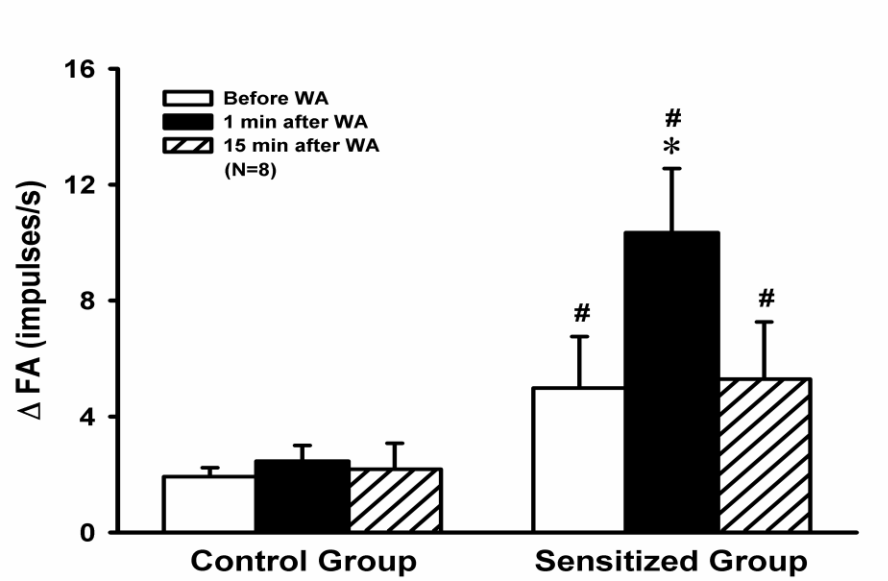

Fig. 3. A comparison of the effects of WA challenge on the average peak response of pulmonary C-fibers to right atrial bolus injection of capsaicin (0.75 mg/kg) between control and sensitized rats. ΔFA, difference between the peak FA (average over 2-s interval) within 3 s after the injection and the baseline FA (average over 20-s interval) in each fiber. Responses were tested before, at 1 min and 15 min after the WA challenge in both control and sensitized rats. Data are means ± SE of 8 rats in each group. **P*<0.05, significantly different from before WA. $^{\#}$*P*<0.05, significant difference when corresponding data between control and sensitized group were compared.

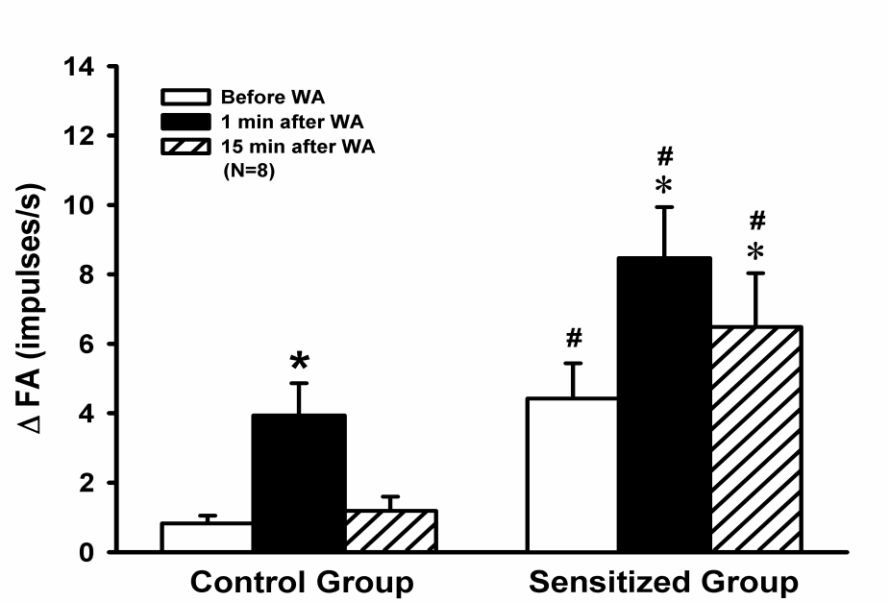

Fig. 4. A comparison of the effects of WA challenge on the average peak response of pulmonary C-fibers to right atrial bolus injection of phenylbiguanide (PBG, 5 mg/kg) between control and sensitized rats. ΔFA, difference between the peak FA (average over 4-s interval) within 5 s after the injection and the baseline FA (average over 20-s interval) in each fiber. Responses were tested before, at 1 min and 15 min after the WA challenge in both control and sensitized rats. Data are means ± SE of 8 rats in each group. **P*<0.05, significantly different from before WA. $^{\#}$*P*<0.05, significant difference when corresponding data between control and sensitized group were compared.

Table 1. Subject Characteristics and AR Symptom Severity Assessments

		AR patients							Healthy subjects					
		#1	#2	#3	#4	#5	#6	#7	#1	#2	#3	#4	#5	#6
Physical data	Age (year)	37	40	21	43	41	30	35	25	28	29	32	29	48
	Sex	M	M	F	F	F	F	F	F	M	F	M	M	F
	Height (cm)	180	175	155	150	160	160	165	170	185	157	173	170	160
	Weight (Kg)	122	104	59	73	73	53	61	58	86	54	61	84	60
A: Nasal	Sneezing	3	2	5	5	2	5	3	1	1	1	1	1	1
	Runny Nose	3	3	4	4	2	5	2	1	2	1	1	2	1
	Congestion (stuffiness)	6	3	6	7	3	5	5	1	1	1	1	2	1
	Itchy Nose	6	3	6	3	2	6	2	1	1	1	1	1	1
	Postnasal Drip	2	4	6	3	NA	6	3	1	2	1	1	1	1
	Total Nasal Symptoms	5	5	6	NA	2	6	3	1	2	1	1	1	1
B: Non-nasal	Eye Symptoms	6	3	4	3	3	3	3	1	2	1	1	1	1
	Throat Symptoms	2	3	6	5	1	NA	3	1	1	1	1	1	1
	Chronic Cough	4	1	5	4	2	6	3	1	1	1	1	1	1
	Ear Symptoms	3	2	6	3	3	3	5	1	1	1	1	1	1
	Headache	5	1	2	5	1	4	4	1	2	1	1	1	1
	Mental Function	1	1	1	5	1	2	1	1	1	1	1	1	1
C: Q.O.L.	Quality of Life	2	5	3	2	5	2	4	7	7	7	7	7	7

AR: allergic rhinitis; Categories A and B: 1 - None; 7 - Unbearably severe; Category C: 1 - None; 7 - Excellent; Q.O.L.: Effect of AR severity on quality of life; NA: Not assessed

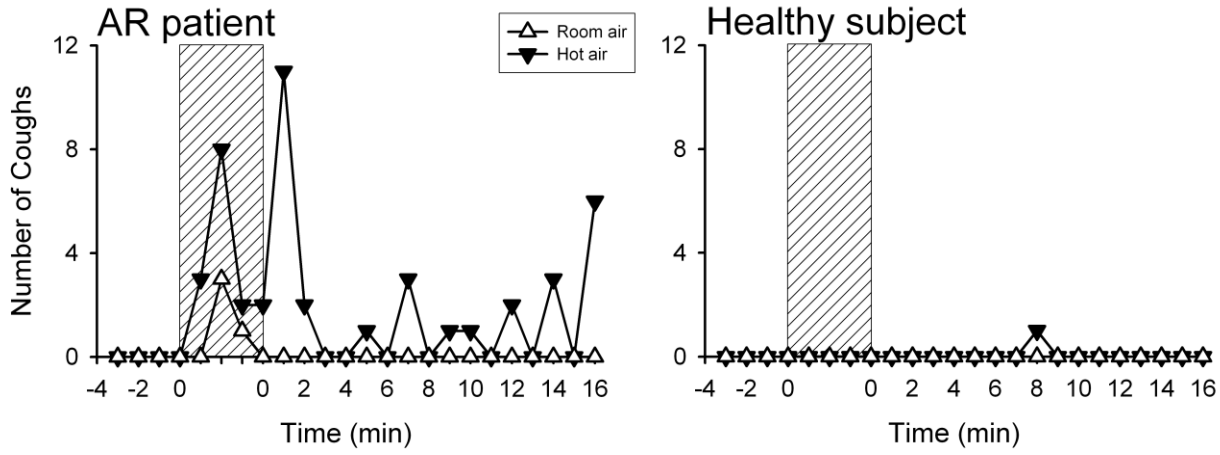

Fig. 5. Representative responses of cough frequency (number of coughs per minute) to hyperventilation of humidified room air (open triangles) and hot air (closed triangles) in a patient with allergic rhinitis (AR; *left panel*) and a healthy subject (*right panel*). During hyperventilation (shaded bars), the subjects breathed at 40% of maximal voluntary ventilation for 4 min of a gas mixture of 4.5% CO2 balance air.

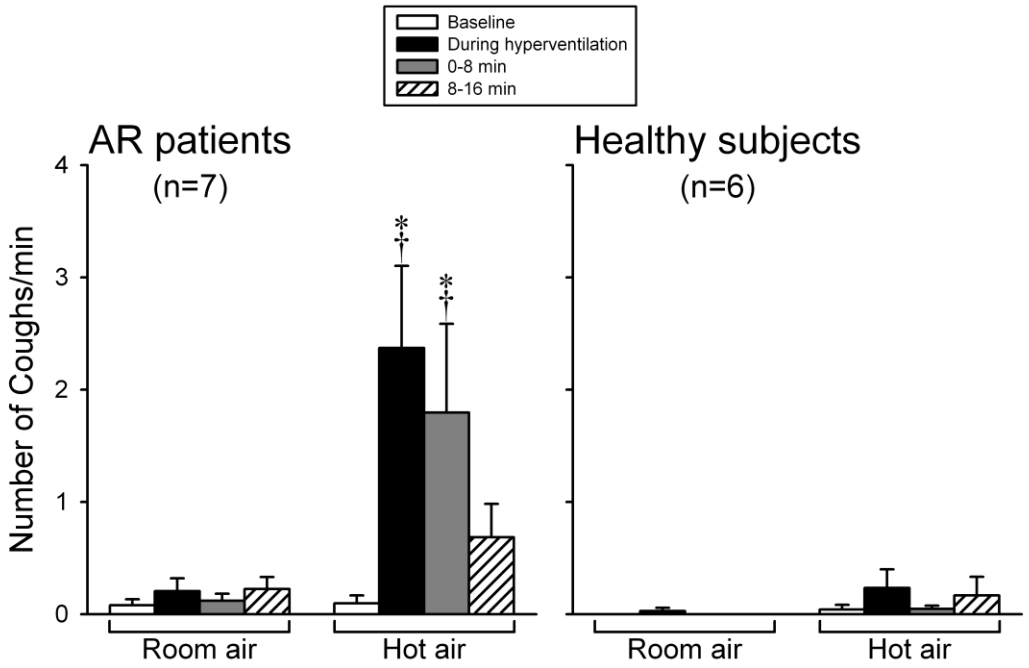

Fig. 6. Comparison of cough responses to hyperventilation of humidified room air and hot air in AR patients (*left panel*; n=7) and healthy subjects (*right panel*; n=6). Cough frequencies were averaged in 8-min durations before and after hyperventilation challenge in each subject. Data are means±SEM. *, significantly different (p<0.05) from the baseline. †, significant difference (p<0.05) between room air and hot air.

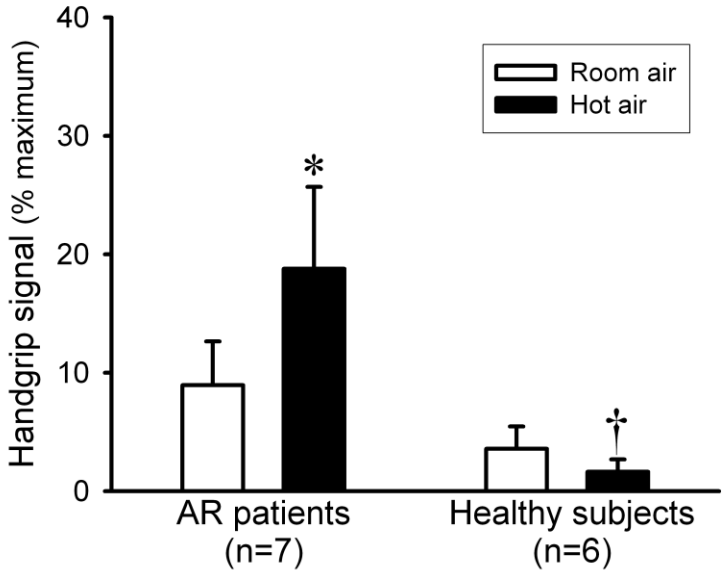

Fig. 7. Airway irritation evoked by hyperventilation of humidified room air (open bars) and hot air (closed bars) in AR patients (n=7) and healthy subjects (n=6). The level of discomfort sensation was expressed by the handgrip dynamometer signal in each individual. *, significant difference (p<0.05) between room air and hot air. †, significant difference (p<0.05) between AR patients and healthy subjects.

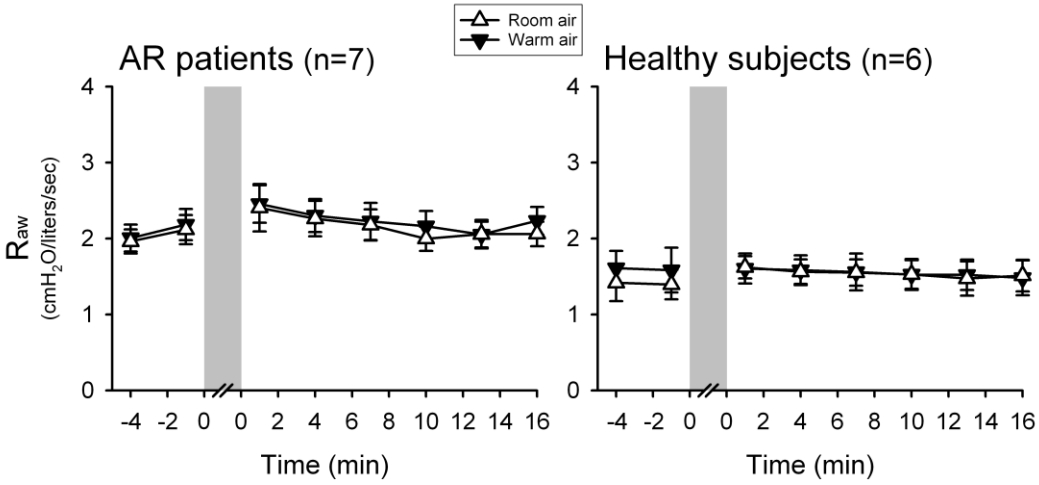

Fig. 8. Comparison of responses of airway resistance (Raw) to hyperventilation of humidified room air (open triangles) and hot air (closed triangles) in AR patients (*left panel*; n=7) and healthy subjects (*right panel*; n=6). Each data point represents Raw averaged over 4 consecutive breaths, and data are means±SEM of all subjects in that group. For detail explanation of hyperventilation challenge (shaded bar), see legend of Figure 5.

KEY RESEARCH ACCOMPLISHMENTS

Our studies completed so far have accomplished the following major milestones::

1) Establish critical information for documenting the pulmonary stresses induced by hyperthermia in healthy individuals and in patients with mild asthma, allergic rhinitis and laryngopharyngeal reflux

2) Document the distinct difference in these airway responses to thermal stress between healthy individuals and patients with inflammatory airway diseases.

3) Characterize the critical role of the cholinergic mechanism underlying the hyperthermia-induced reflex bronchoconstriction.

4) Demonstrate the involvement of TRPV1-sensoty nerves in eliciting these airway response to thermal stress.

5) All these new information have established important information for detecting the susceptibility to heat stress in soldiers with underestimated or overlooked airway hypersensitivity, such as in individuals with mild asthma or allergic rhinitis.

REPORTABLE OUTCOMES

1. Publications

In the last twelve months, These full papers have been published or submitted for pulication; these papers reported the findings obtained in the studies supported either in full or in part by this TATRC:

Lee, L.-Y., Q. Gu, F. Xu, and J.L. Hong. Acid-sensing by airway afferent nerves. Pulm. Pharmacol. Ther. 2013 (Invited review; Epub ahead of print: PMID 23524016)

Lin, R.L., Y.J. Lin, M.J. Geer, R. Kryscio, and L.-Y. Lee. Pulmonary Chemoreflex Responses Are Potentiated by Tumor Necrosis Factor Alpha in Mice. J. Appl. Physiol. 2013 (Epub ahead of print: PMID 23539315)

Hsu, C.C., Y.S. Lin, R.L. Lin and L.-Y. Lee. Bronchoconstriction induced by increasing airway temperature in ovalbumin-sensitized rats: role of tachykinins. J. Appl. Physiol. 2013 (Epub ahead of print: PMID 23845978)

Dicpinigaitis, P.V., G.A. Fontana, L.-Y. Lee, and M. Tatar. Summary of papers presented at the 2013 Seventh International Cough Symposium. BioMed Central Cough J. 2013 (Epub ahead of print: PMID: 23639195)

Hsu, C.C., R.L. Lin, L.-Y. Lee and Y.S. Lin. Hydrogen sulfide induces hypersensitivity of rat lung vagal neurons: role of TRPA1 receptors. Am. J. Physiol. : Reg. Int. Comp. Physiol. 2013 (Epub ahead of print: PMID 23842678)

Khosravi, M., P. B. Collins, R.-L. Lin, D. Hayes, and L.-Y. Lee. Cough response triggered by hyperventilation of humid hot air in patients with allergic rhinitis. ???? (Submitted, 2013)

Lee, L.-Y., and J. Yu. Sensory nerves in lung and airways. Comprehensive Physiology ed. by G.S. Mitchell, Am. Physiol. Society, 2013 (Invited review; in press)

2. Employment Generated by this TATRC Contract

Salaries of the employees listed below are paid in part or in full with the funds provided by this research contract during 2012-2013:

Lu-Yuan Lee, Ph.D., Principal Investigator (30% effort)
Mahdi Khosravi, M.D., Co-investigator (20% effort)
Paul B. Collins, B.S., RRT, Supervisor of Pulmonary Function Laboratory (10% effort).
Richard Kryscio, Ph.D., Co-investigator (3% effort), Consultant for Biostatistics (3% effort)
Marcus Geer, B.S., Lab Technician (100% effort)
Robert Morton, Part-time Senior Research Analyst (20% effort)
Ruby Lin, B.S., Graduate Assistant (100% effort)
Reyno Tapia, Undergraduate Part-time Lab Assistant (20% effort; newly hired to work on this project).

CONCLUSIONS

Based upon the results obtained from the studies performed in our lab in the last twelve months, we can reached the following conclusions:

1) Increasing airway temperature evoked a pronounced and reversible increase in airway resistance in sensitized rats and tachykinins released from the vagal bronchopulmonary C-fiber endings were primarily responsible.

2) In an animal model of asthma (Brown-Norway rats sensitized by ovalbumin), chronic allergic inflammation sensitization increases the excitability of pulmonary C-fibers to chemical stimuli including both TRPV1 and non-TRPV1 activators. The hypersensitivity is further enhanced by an increase in the airway temperature.

3) Hyperventilation of humid warm air triggered vigorous cough response and respiratory discomfort in patients with allergic rhinitis, indicating the involvement of the airway sensory nerves. Chronic inflammation in the upper airways may have contributed to an up-regulation of the sensitivity and/or expression of TRPV1 in these sensory nerves.

4) Preliminary studies showed that vigorous cough response accompanied by high frequency of vocal fold adductor motion were triggered during hyperventilation with hot humid air in patients with laryngopharyngeal reflux, and these responses were absent in in healthy control subject.

These observations indicated that further studies will be required to determine whether the effect of hyperthermia is primarily mediated through an activation of the temperature-sensitive TRPV1 channel expressed on vagal bronchopulmonary C-fibers, and if TRPV1 expression is up-regulated in the airway mucosa of patients with chronic inflammation.

APPENDIX

Electronic copies (reprints) of the following publications supported either in full or in part by this TATRC project are attached to this Annual Progress Report:

Lee, L.-Y., Q. Gu, F. Xu, and J.L. Hong. Acid-sensing by airway afferent nerves. Pulm Pharmacol Ther. 26:491-7, 2013. http://dx.doi.org/10.1016/j.pupt.2013.03.010

Lin, R.L., Y.J. Lin, M.J. Geer, R. Kryscio, and L.-Y. Lee. Pulmonary Chemoreflex Responses Are Potentiated by Tumor Necrosis Factor Alpha in Mice. J. Appl. Physiol. 114:1536-43, 2013. http://dx.doi.org/10.1152/japplphysiol.01301.2012

Hsu, C.C., Y.S. Lin, R.L. Lin and L.-Y. Lee. Bronchoconstriction induced by increasing airway temperature in ovalbumin-sensitized rats: role of tachykinins. J. Appl. Physiol. 115:688-96, 2013. http://dx.doi.org/10.1152/japplphysiol.00491.2013

Hsu, C.C., R.L. Lin, L.-Y. Lee and Y.S. Lin. Hydrogen sulfide induces hypersensitivity of rat lung vagal neurons: role of TRPA1 receptors. Am. J. Physiol. : Reg. Int. Comp. Physiol. 305:R769-79, 2013. http://dx.doi.org/10.1152/ajpregu.00202.2013

Khosravi, M., P. B. Collins, R.-L. Lin, D. Hayes, and L.-Y. Lee. Cough response triggered by hyperventilation of humid hot air in patients with allergic rhinitis. Clin. & Exp. Allergy (Submitted in October 2013)

Lee, L.-Y., and J. Yu. Sensory nerves in lung and airways. Comprehensive Physiology ed. by G.S. Mitchell, Am. Physiol. Society, 2013 (Invited review; in press) http://dx.doi.org/10.1002/cphy.c130020